In The United States Patent and Trademark Office

In re U.S. Patent Application of: **Jeffrey Stavenhagen *et al.***	Examiner: **Crowder, Chun**
Serial No. **10/754,922**	Group Art Unit: **1644**
Filed: **January 9, 2004**	Attorney Docket No.: **1301.0004C**
For: **Identification and Engineering of Antibodies with Variant Fc Regions and Methods of Using Same**	Confirmation No. **8663**

Filed via EFS-Web
Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Jeffrey Stavenhagen, Ph.D. Pursuant to 37 C.F.R. §1.132

Sir:

I, **Jeffrey Stavenhagen, Ph.D.**, hereby declare as follows:

1. I believe that I am an original and joint inventor of the subject matter which is claimed and for which a patent is sought on the above-described patent application, the specification of which was filed on January 9, 2004, as Application Serial No. 10/754,922 (the "Application").

2. I have reviewed, and am familiar with, the Application (including its pending claims) and the Official Action of the United States Patent and Trademark Office that issued on April 19, 2007 with respect to the Application. I have provided this Declaration in order to provide evidence addressing the conclusions of the Examiner that the Application, as of its effective filing date failed to provide those of ordinary skill with sufficient guidance:

   (A) to enable the full scope of claims directed to molecules comprising "*a polypeptide comprising a variant Fc region*" having at least an amino acid

modification at position 396 relative to a wild-type Fc region, and exhibiting increased affinity relative to a polypeptide comprising said wild-type Fc region; and

(B) regarding amino acid substitutions that may alter antibody function to support the presently claimed invention, to enable the full scope of the claims.

3. In 1984, I received a B.S. degree in molecular biology from Carnegie-Mellon University (Pittsburg, PA), and in 1989, I received a Ph.D. degree in molecular Genetics from Columbia University (New York, NY). From 1989 to 1990, I was a post-doctoral research fellow at INSERM in Paris, France. From 1990 to 1996, I was a post-doctoral research fellow at the Fred Hutchinson Cancer Research Center (Seattle, WA). From 1996-2000, I was an Assistant Professor of the University of Dayton (Dayton, OH). I have been employed at MacroGenics, Inc. (Rockville, MD), the assignee of the present application, since 2000, and have served as MacroGenic's Associate Director, Molecular Immunology from 2004 to 2006. I am currently the Director, Molecular Immunology of MacroGenics, Inc. I therefore submit that I am qualified to provide an opinion as to the ability of those of ordinary skill to practice the full scope of the presently claimed invention as of the effective filing date of the Application. I am employed by the Assignee of the Application.

4. The pending claims of the Application are directed to antibodies and other polypeptides that comprise a "variant" Fc region. The Fc region is termed a variant region because it differs in amino acid sequence from that of a naturally occurring (wild-type) Fc region by comprising at least an amino acid modification at position 396 relative to said wild-type Fc region[1]. The variant Fc region of encompassed molecules must also bind to the cellular receptor for the Fc region of IgG immunoglobulins (termed "FcγR") with increased affinity relative to the affinity of binding of the naturally occurring (i.e., "pre-variant") Fc region. As disclosed in the Application (see, generally pages 5-10), and as would be recognized by those of

---

[1] The numbering of amino acid residues follows the standard system of nomenclature (i.e., "the EU index as in Kabat") used by those of ordinary skill in this field.

ordinary skill in light of the Application, a molecule with increased affinity for the FcγR is of very substantial utility in the treatment of numerous disorders, including: **cancer** (by improving the specificity of antibodies directed against cancer-associated antigens), **inflammatory disease** (by competing with undesired, naturally elicited antibodies for FcγR binding and thereby attenuating the undesired immune response that characterizes an inflammatory process, and **infectious disease** (by improving the ability of antibodies to elicit an immune response against antigens associated with the presence of infectious agents).

5. The pending claims of the Application thus reflect the recognition that the ability of an Fc region to bind to a cellular receptor residue can be augmented by altering the amino acid residue that is naturally present at position 396 of the Fc region. The specification exemplifies this recognition by disclosing the production of molecules having substitutions of leucine (an uncharged non-polar amino acid) and histidine (a charged, polar amino acid) for the proline residue naturally found at position 396. As disclosed in the Application (page 183; **Table 10**) either change was shown to be sufficient to cause the variant Fc region to bind to the FcγR with increased affinity (relative to the affinity of a molecule comprising an Fc region with proline at position 396).

6. It is my opinion that the Application would have fully enabled those of ordinary skill in the art as of the effective filing date of the Application to practice the full scope of the claimed invention. I draw this conclusion because I find that the Application fully enabled such individuals to make and use:

   A. Fc variant regions having ***any*** substitution at position 396;
   B. Fc variant regions having ***any*** substitution at position 396 and ***also*** any additional substitution(s) at any position(s) ***other than*** position 396; as well as
   C. ***Any*** polypeptide containing such Fc variant regions (i.e., not merely an Fc region or an antibody containing an Fc region);

   and that such enablement permits the full practice of the presently claimed invention. The bases for these conclusions are provided below.

A. Enablement As To Fc Variant Regions Having Any Substitution At Position 396

(1) I believe that it is important to emphasize the significance of the Application's disclosure of the significance of variations at Fc position 396. It is my opinion that, absent such disclosure by the Application, those of ordinary skill would not have known which of the 198 amino acid residues of the Fc region would have needed to be altered in order to achieve an Fc region having increased FcγR binding affinity, or indeed whether it was even possible to attain such a desired variant.

(2) In light of the prior absence of information relating to Fc residues that increased FcγR binding affinity, the Application used a functional genetic screening approach to identify the relevant Fc residue from a random library of Fc mutants. This approach surmounted both the above-mentioned prior deficiencies: at once establishing that such desired Fc variants ***could*** be produced, and ***guiding*** those of ordinary skill to the selection of specific Fc regions containing variations at position 396.

(3) It is my conclusion that, in light of such explicit disclosure by the Application of the production of 396 leucine or histidine Fc variants, the production of ***any*** of the remaining **17** possible position 396 variants would have involved only mere routine experimentation. In this regard, I note that, as of the effective filing date of the application, the amino acid and nucleotide sequences of naturally occurring Fc regions were known to those of ordinary skill. Indeed, the Application provides deposited clones of suitable Fc regions (see Paragraph No. **0055**). Paragraph No. **00177** of the Application teaches that:

"Mutagenesis may be performed in accordance with any of the techniques known in the art including, but not limited to, synthesizing an oligonucleotide having one or more modifications within the sequence of the Fc region of an antibody or a polypeptide comprising

an Fc region (*e.g.*, the CH2 or CH3 domain) to be modified. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 30 to about 45 nucleotides or more in length is preferred, with about 10 to about 25 or more residues on both sides of the junction of the sequence being altered. A number of such primers introducing a variety of different mutations at one or more positions may be used to generate a library of mutants."

(4) Thus, in light of the Application's disclosure, I conclude that the production of a position 396 Fc region variant having ***any*** desired amino acid residue in place of the naturally occurring proline residue, would have involved no more than employing the well-known Genetic Code to fashion oligonucleotides encoding the desired Fc region, and the use of such oligonucleotides to produce the desired Fc variant using any of a battery of well-known techniques. It is my conclusion that, in light of the Application's disclosure, such activities could have been readily and successfully undertaken by those of ordinary skill as of the effective filing date of the Application. Thus, I have concluded that the Application fully enabled those of ordinary skill to make and use Fc variant regions having ***any*** substitution at position 396.

B. Enablement As To Fc Variant Regions Having Any Substitution At Position 396 And Also Any Additional Substitution(s) At Any Position(s) Other Than Position 396

(1) It is my conclusion that, given the Application's above-described enablement of improved Fc variants having *any* of the 19 possible position 396 variants, enablement as to improved Fc variants possessing *additional* amino acid sequence modifications merely involves providing those of ordinary skill with the means to efficiently produce, recognize and recover such variants.

(2) In this regard, the Application provides *extensive* general disclosure (see Paragraph Nos. **00162-00224**) and detailed Examples (see Paragraph Nos. **00358-00440**) describing the successful use of phage display and other techniques to produce and screen libraries of Fc variants for members that exhibit increased binding affinity for FcγR, and which have alterations *in addition to* an alteration at Fc position 396.

(3) As disclosed in the Application, such methods permit the facile production and simultaneous screening of hundreds of millions of Fc variant clones (see, for example, Paragraph No. **00397**), and actually resulted in the identification of a substantial number of exemplary encompassed Fc variants.

(4) Thus, it is my conclusion that the description of library production and screening procedures provided by the Application, coupled with their actual demonstrated use to produce encompassed Fc variants would have fully enabled those of ordinary skill as of the effective filing date of the Application to have made and used Fc variant regions having *any* substitution at position 396 and also *any* additional substitution(s) at any position(s) other than position 396 using only mere routine experimentation.

C. Enablement Regarding *Any* Polypeptide Containing Such Fc Variant Regions

(1) It is my conclusion that, given the Application's above-described enablement of improved Fc variants having *any* of the 19 possible position 396 variants, alone or in concert with *any* additional substitution(s) at any position(s) other than position 396, the Application fully enables the production of polynucleotides encoding such Fc variants, and thus enabled those of ordinary skill to make and

use *any* polypeptide containing such Fc variant regions as of the effective filing date of the Application.

(2) In this regard, the Application discloses that the encompassed variant Fc regions are polypeptides that may be conjugated to a wide variety of molecules (and thus such molecules would comprise "a polypeptide comprising a variant Fc region"). Such molecules include: monoclonal, bi-specific, multi-specific, human, humanized, chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, disulfide-linked Fvs, and fragments containing either a VL or VH domain or even a complementary determining region (CDR) that specifically binds an antigen, in certain cases, engineered to contain or fused to an FcγR binding region (see, e.g., Paragraph No. **0050);** polypeptide cellular ligands (see, e.g., Paragraph No. **00144**); engineered human or humanized antibodies (see, e.g., Paragraph No. **00148)**; polypeptide toxins (see, e.g., Paragraph No. **00154**); polypeptide marker sequences (see, e.g., Paragraph No. **00155**), enzymes (see, e.g., Paragraph No. **00157**), antibody conjugates (see, e.g., Paragraph No. **00161**). Methods of producing such molecules via recombinant DNA technology or other means were extensively known to those of ordinary skill in the art as of the effective filing date of the Application.

(3) Thus, given the Application's enablement of polynucleotides encoding encompassed Fc variants, it is my conclusion that the production of polypeptide conjugates comprising such encompassed Fc variants would involve mere routine experimentation well within the purview of those of ordinary skill as of the effective filing date of the Application.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and

the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully Submitted,

Date: **July 18, 2007**

/Jeffrey Stavenhagen/
Jeffrey Stavenhagen, Ph.D.